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FURTHER STUDIES OF DISEASES AFFECTING MOOSE

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"SIDEWAYS HOLDING" OF HEAD SHOWN BY MOOSE NO. 13

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INTRODUCTION

Systematic study of disease in wild animals forms one of the latest branches of animal ecology. It is safe to say that many species suffer from outbreaks of disease no less severe than those which attack domestic animals. It is important to know that, under natural conditions, the fluctuations in numbers are not attributable to human interference or to diseases contracted from domestic animals maintained in or near the wild-animal haunts. Such fluctuations are caused either by disease or by unknown factors. Fluctuation in numbers occurs somewhat regularly in some species. It cannot be said that a cyclic decimation of moose has ever occurred in Minnesota. As far as can be determined, moose were present in Minnesota as early as 1860 (1). The last legal hunting season for moose was in 1922. Scarcity in numbers made it necessary to protect them by law.

More reports of sick and dead moose are received now than formerly because areas inhabited by moose are more accessible, and the general public has become more interested in the conservation of wild life. A moose seen lingering near a highway or logging road and failing to show fear of man is reported to the proper game wardens.

Diseases that may affect moose are important not only because the health and welfare of the animal itself are concerned, but because of public health and for economic reasons. The study of such diseases, however, is beset with many difficulties. It is possible to observe a healthy individual in the wilds only for a very short time. Our study has been confined to cases showing abnormal behavior. It has not been deemed advisable to destroy any healthy individuals.

The results of examinations of 11 moose have been reported (2). In that report it was said: "The knowledge of diseases of wild animals as they occur under natural conditions is very meager. It is possible that an entirely new and hitherto undescribed pathogen is the cause of disease among moose; also that the conditions studied are brought about

[1] Throughout the work here reported the Division of Entomology and Economic Zoology has co-operated in the parasitological aspects of the study, special acknowledgment being due Assistants C. G. Fredine, F. G. Wallace, and Wm. L. Jellison. We also gratefully acknowledge assistance rendered by E. F. Waller, of the Division of Veterinary Medicine, and by members of the Department of Conservation of the State of Minnesota.

through several forces co-operating to reduce the vitality of moose and produce the symptoms observed."

Since that report was presented, we have observed five live moose, holding autopsies after slaughtering them. Moving pictures of these animals were taken and a visible record showing the symptoms and actions is, therefore, available. The carcass of an additional moose and the head and neck of another were also received for examination.

HISTORY AND HABITS

It is difficult to obtain authentic reports in regard to disease among moose. Surber (1) has stated that 70 years ago moose were seen occasionally as near as 80 miles north of St. Paul and Minneapolis. They shun civilization, and now they are found in the area north of Lake Superior. The western limit of their range in Minnesota can be placed arbitrarily at the Lake of Woods. Old settlers and trappers say that moose are continually shifting back and forth across the Canadian border. Moose are more solitary in their habits than deer. They congregate along certain lakes in the summer when insects are most numerous, but the following season are usually found in a different place. In searching for information as to first evidences of disease among moose, one must depend on information obtained from old settlers and hunters. Statements from such persons indicate that moose infested with ticks have been observed for at least 20 or 25 years. The moose is the preferred host of the tick, *Dermacentor albipictus* Packard, and infestation is common.

Ticks found on elk in Itasca State Park were identified in 1915 by Howard (3) as *Dermacentor albipictus* Packard. These elk had been imported from Montana several years before. Howard's report is the earliest authentic record we can find of the presence of this parasite in Minnesota, and it is likely that this tick was present in Minnesota before the importation of elk from Montana.

The finding of dead and sick moose is not confined to any particular section of northern Minnesota, and it is impossible to estimate the extent of mortality among moose in the state. There is no census of moose, and no doubt many dead animals never come to the attention of wardens, being consumed by predators. But carcasses are being found by game wardens more frequently now than in former years. The writer is informed that more carcasses of yearling moose are found than of older animals. We are inclined to believe, as before, that the losses are increasing. For the last three years we have made the most thoro examination possible of every sick and dead moose reported to us by the State Conservation Department,—three or four each year.

Table 1

Data on Material Available for Examination

Moose No.	Date	Material secured for examination	Condition at time of necropsy	Examinations conducted
12	February 17, 1933	Entire carcass	Found dead	Parasitological Bacteriological Pathological
13	March 21, 1933	Entire carcass	Destroyed	Hematological Parasitological Bacteriological Pathological
14	April 10, 1933	Entire carcass	Destroyed	Hematological Parasitological Bacteriological Pathological
15	September 12, 1933	Entire carcass	Destroyed	Hematological Parasitological Bacteriological Pathological
16	October 11, 1933	Entire carcass	Destroyed	Hematological Parasitological Bacteriological Pathological
17	January 12, 1934	Entire carcass	Destroyed	Hematological Parasitological Bacteriological Pathological
18	January 12, 1934	Head and neck	Found dead	Parasitological Pathological

SYMPTOMATOLOGY

It is difficult to approach a healthy moose except when flies and other insects are numerous. At such times, moose will migrate to lakes and streams containing aquatic plants on which they feed, and, while they are feeding in the water, one using a canoe may occasionally approach within a few yards. But when a moose observes the approach of man, it will usually rush into the wilderness. The animals detect the presence of intruders chiefly through the senses of smell and hearing. If they are warned by sound, they immediately face the wind in order to locate definitely the intruder. Once they discover the direction from which danger is approaching they escape by going in the opposite direction. They have been seen to swim several miles in order to escape. The most distinctive feature observed as common to all sick moose is that they do not show the usual fear of man. Moose No. 15 was an exception to this rule. This bull had been lingering for several weeks within a small area along a logging railroad. At the time of our arrival he was not to be seen. His bed was found after a short time, and he was

tracked through the forest for several hours. Finally he returned to the railroad track, and from then on it was possible to drive him away for only a short distance. Moose No. 16 was rather aggressive. She remained constantly near a settler's home and the occupants were afraid to leave the house. Several times she charged one of our party who was attempting to drive her in order that motion pictures could be taken. She appeared to be blind. The motion pictures show that she walked against a post supporting a barbed-wire fence. After encountering the fence, she turned and walked away. How she was able to chase a man for 75 yards and follow closely cannot be explained unless it was by scent alone.

Two of the moose (Nos. 13 and 15) showed symptoms that were not seen in others. Moose No. 13 held her head in a lateral position at times, the lower part of the head being turned to the right. At other times she held her head in a normal position. The left ear drooped sometimes, and then again it was held in the usual manner. The other moose, No. 15, was blind in the right eye, and carried its head in a similar manner, but turned in the opposite direction—to the left. The head could be handled in a normal manner. In observing these two animals, one was impressed with two possibilities: either the presence of a tick or an infection located in the middle or inner ear.

One thing common to all diseased moose is their determined effort to remain near the place in which they are found. They can be driven away, but they will run in a circle to the place from which they started. If they are not emaciated, they can be driven for several hours without exhibiting any marked evidence of exhaustion. Several of those that were emaciated, tired very quickly when driven through deep snow. If they are not disturbed by actual driving, they simply stand still, face the wind, and maintain a watchful attitude. Only one exception to this (No. 16) was observed. In this instance, if undisturbed, the animal walked aimlessly—sometimes in small circles.

Emaciation was common to four moose studied (Nos. 12, 14, 16, and 18). The other three were in a good state of nutrition. Normal moose carry less flesh in the spring than in the fall. Food is not so abundant in winter, thus in spring an animal would be more or less emaciated. In the fall, however, a normal moose is usually sleek and fat and has a black color.

Cahn and his associates (4) state: "The diseased moose behave strangely. They become extremely active and are constantly on the run; they race blindly through the woods, bumping into trees and falling over other obvious obstacles, or they take to the open spaces of the lake. If there is ice, they wander in circles, aimlessly, blindly; if there is open water, they swim about without rime or reason, growing weaker

and weaker until they drown. If they do not meet a watery grave, they eventually exhibit a form of paralysis which causes them to break down in the hind quarters, sometimes in the fore quarters."

Contrary to the reports by Cahn and his associates (4), paralysis has never been noted as a symptom in the moose we have observed. One yearling male moose (No. 14) was nearly dead when first observed. This animal was extremely emaciated and was hardly able to stand. Wherever he lay down, the snow was stained red by blood from tick-bite wounds and from the crushing of engorged ticks. When led, he was able to walk with an unsteady gait. One moose (No. 17), when first observed, was lying on her abdomen with the head extended as tho asleep. No attention was given to our presence until we approached from the windward side. She then arose without difficulty, gazed about, defecated and urinated. No attempt was made to escape. It was necessary to step aside as she approached. After struggling through the snow (about three feet deep) for a short time, she stumbled and fell. Having fallen among trees and windfalls, she got up with difficulty. She weakened rapidly and fell more often, as we drove her in order to obtain moving pictures. This is the only animal observed that repeatedly threw herself over backwards. This was accomplished in a peculiar manner. When she walked or ran into a mass of windfalls, she would climb up with her front limbs, and fall over backwards.

The skin of moose examined during late winter or early spring frequently shows denuded areas. Presumably the irritation produced by large numbers of ticks is responsible for their rubbing against trees or windfalls. Therefore, it is not uncommon to find no hair on the skin in many places, particularly on the shoulders, neck, ventral and lateral sides of the abdomen, the pectoral and inguinal regions, the medial surface of the femoral region, and the posterior surface of the hind limbs. The skin in these areas has a thick, leathery appearance similar in some respects to that of cattle affected with mange. Dried blood was found adhering to the skin. Blood may sometimes be seen along the trails and on windfalls frequented by tick-infested moose.

Rectal and intra-abdominal temperatures were taken in all instances when a moose was destroyed. The maximum temperature found was 102.4° F. The following is a record of the temperatures:

Moose No. 13, 100.8° F. Rectal temperature.
Moose No. 14, 102.4° F. Intra-abdominal temperature.
Moose No. 15, 102.0° F. Rectal temperature.
Moose No. 16, 100.5° F. Rectal temperature.
Moose No. 17, 101.4° F. Rectal temperature.

NECROPSY FINDINGS

We have examined by necropsy seven moose since the first publication was prepared. It was possible, in one instance, to obtain only the head and part of the neck. The entire carcass of Moose No. 12 was received for examination. Death was due, no doubt, to an accidental injury. The left frontal bone was shattered completely. The fragments were removed easily with rat-tooth forceps, and the muscles covering the bone were badly bruised.

The dates when these autopsies were performed were more widely distributed throughout the year than those previously reported (2). Post-mortem examinations were made in January, February, March, April, September, and October. Therefore we were afforded a better opportunity to note the tick infestation present and to determine whether the host was infested with larval, nymphal, or adult ticks, *Dermacentor albipictus* Packard. No ticks were found on one moose (No. 16). All others were tick-infested. Some moose were harboring thousands of ticks, while several others were practically tick-free. According to our data, the larval ticks were found in January and February; the nymphal ticks in January, February, March, April, and September, and the adult ticks in March and April. All moose autopsied in the latter part of the winter have been found heavily infested chiefly with adult ticks.[2]

The skin was removed from each carcass. It was then placed in a heavy canvas sack so that no ticks could escape. After a skin was brought to the laboratory, the hair was clipped off. Clipping is particularly important when the host is infested with only a limited number of immature ticks. In one instance only nine nymphal ticks were collected from an entire moose hide.

On opening the carcasses, we found that three of the moose (Nos. 13, 15, and 17) were in an excellent state of nutrition. An abundant supply of fat was found distributed in the usual locations. This finding is contrary to our previous report (2). The other four carcasses were emaciated. The degree of emaciation varied. Usually when an autopsy is performed in March and April, the muscles are reduced in size and the fat deposits are depleted.

Lungs.—The lungs in four animals (Nos. 13, 14, 15, and 16) were infested with nematodes identified as *Dictyocaulus hadweni* Chapin, 1925. The infestation was severe in one case (No. 14); in others only five to ten were found. In case of heavy invasion, both principal lobes were involved. In the others, the parasites were recovered from only one lobe. Congestion of the lungs was noted in the areas infested with the nematode.

[2] The white tail deer, *Odocoileus virginianus*, is also known to be a host of the tick, *Dermacentor albipictus* Packard. The author found two deer infested with this ectoparasite.

The lungs of one moose (No. 15) contained 20 cysts. These were the cystic stage of the *Echinococcus granulosus*. This has been confirmed by Wallace (2, 5). The cysts were found upon palpation to be firm. Such cysts are usually cylindrical but may be decidedly elongated. They are found scattered throughout the parenchyma and feel like a solid foreign object. They always contain a light-colored fluid. Upon close examination, this fluid is found to contain countless numbers of small granules resembling grains of fine sand.

Law and Kennedy (7), of the Ontario Government Fur Farm, recently conducted a post-mortem of an adult moose and found a heavy infestation of *Echinococcus granulosus* in the lungs. The animal was weak and showed little inclination to run from the hunter. The normal tissue of the lungs was almost completely replaced with cysts containing numerous larval scolices. The moose was not infested with *Dermacentor albipictus*. (The season of the year when the autopsy was performed was not stated.)

Wallace (5) has listed the parasites found in the examination of 14 moose in Minnesota. These include the 11 cases that were reported in our previous publication (2) and three of the seven cases now being reported. The literature pertaining to these parasites has been reviewed by Wallace.

Three cestode cysts, *Cysticercus* sp., were found imbedded in the muscular portion of the diaphragm (two specimens) and in the lungs (one specimen). The cysts were collected from Moose No. 16. These were examined by Jellison (8) who noted that they were considerably smaller than those of *Taenia hydatigena* Pallas found in deer and moose. He fed them to an experimental fox but on necropsy 100 days later no cestodes were recovered. Wallace had previously noted similar cysts in the heart muscle of two other moose and had called attention to their resemblance to those of *Taenia solium*. In view of the food habits of moose, it is highly improbable that they were the cysts of *Taenia solium*. Ransom (14) pointed out that similar cysts in mutton had often been reported as identical with those found in pork, but in reality were those of *Taenia ovis*. Further experimental work is necessary to determine whether this species may be found also in moose.

Liver.—Parasitic cysts near the surface of the liver were collected from four cases (Nos. 13, 14, 15, and 17). The number of cysts found varied from 3 to 15. These have been identified as *Cysticercus tenuicollis,* the mature form of which, *Taenia hydatigena,* parasitizes the dog, the wolf, and related carnivora. Cysts obtained from Moose No. 17 have been fed to an experimental dog free of intestinal parasites. The latter was autopsied 67 days later and two tapeworms were recovered. C. G. Fredine has identified these cestodes as sexually imma-

ture *Taenia hydatigena*. In this moose similar cysts were found imbedded in the mesentery. Flukes were recovered from the liver of Moose No. 12. Wallace (5) identified these as *Fascioloides magna* Bassi. Wallace (5) says: "Although this parasite has been reported many times from members of the Cervidae in Europe and America, it apparently has not previously been found in the moose. The largest measured 27 mm. in length and 14 mm. in width while the smallest was 7 mm. in length and 4 mm. in width . . . . This is much smaller than the *Fascioloides* ordinarily found in deer and cattle, but nevertheless the morphological features seem to be identical with those of the larger specimens."

Spleen.—This organ in Moose No. 17 was very much enlarged. It was 10 inches long and elliptical in shape. Its maximum thickness was three inches. Normally this organ is about five inches in length and does not exceed an inch in its greatest thickness. It was also very hyperplastic. While being removed, it was accidentally incised and blood spurted from the incision. The spleen appeared to be normal in each of the other moose.

Rumen.—The rumen in every case was well-filled with food, the contents varying markedly with the time of year and the locality. In the fall, especially when moose are found near deserted lumber camps, considerable clover and timothy are found in the rumen. In the spring the contents consist of balsam, birch, brush and popple twigs. Moose will dig through deep snow to obtain frozen plants. The rumen contents were moist. *Paramphistomum cervi* (Zeder) was found in one case (No. 15). The largest number of parasites was found attached to the mucous membrane adjacent to the fold between the rumen and reticulum. Ingesta in this area contained a large number of flukes. The presence of these flukes is not considered to be of pathological significance. In our prevous report, this endoparasite was found in April and May; in this instance the autopsy was in September.

Intestines.—Congestion of the mucous membrane of the small intestine seems to be a constant and possibly a normal finding. The congestion increases in the posterior part of the bowel. Nematodes were found in the small intestines of three moose (Nos. 13, 15, and 17). The nematodes were identified as *Nematodirella longispiculata* York and Maplestone (12). Cestodes identified as belonging to the genus Moniezia were found in the intestines of two moose (Nos. 13 and 16). In one instance only fragments of the parasite were recovered. From the small intestine of Moose No. 16, over 18 meters (measured after formalin fixation) of tapeworm sections, representing at least three individual worms, were recovered. These definitely belong to the genus Moniezia Blanchard, various species of which have been reported from

many members of the Cervidae, but apparently not for *Alces alces* of Europe or *Alces americanus*. This material is in good condition and stained mounts have been prepared. A specific diagnosis, on the basis of the literature alone, is questionable (8). Specimens have been sent to the Zoological Division of the Bureau of Animal Industry for identification.

A single specimen of another nematode found while examining the intestines of Moose No. 16 has been determined as a mature female of *Setaria labiato-papillosa* Alessandrini. It has not been reported previously for moose, but is known to occur in other members of the Cervidae (9). Species of the *Setaria* usually infest the peritoneal cavity and may be found in other serous cavities of the body (6).

Brain.—Congestion of the brain seems to be a rather constant necropsy finding. Such congestion was found in Moose Nos. 15 and 17.

A careful examination was made of the entire auditory tract of two moose (Nos. 13 and 15) that held their heads in an abnormal manner. No evidence of otitis media was found. There was no tick or other obstruction in the ear canal.

Eyes.—In one case (No. 16) a marked opacity of the cornea of the left eye was observed. Upon examination a live nematode measuring 9.3 cm. long was found in the anterior chamber of the left eye. A similar nematode was found in the posterior chamber of the right eye where it was entangled in a loose mass of abnormal tissue. These may be males of *Setaria labiato-papillosa*, but due to immaturity or abnormal development are lacking in specific characters (8).

In horses specimens of *Setaria equina* are occasionally found in the chambers of the eye, and, according to Kaupp (6), may produce an opacity of the cornea and an inflammation of the eyeball.

One other moose (No. 15) was blind in the right eye. A scar approximately one-half inch in length was found upon the corneal surface. The cornea was opaque and very much thickened. Apparently this change of the cornea was the result of mechanical injury.

No gross pathological changes were found in the heart, kidneys, bladder, recticulum, omasum and abomasum in any of the moose.

BLOOD STUDIES

Blood specimens were collected from all the moose that were destroyed. The jugular vein was exposed and punctured as soon as possible after death from shooting, and blood smears were prepared on glass slides immediately. In extremely cold weather it is necessary to keep the slides near one's body for warmth; otherwise, the small amount of blood used in making a blood smear freezes before it can be spread in a thin film. A quantity of blood was collected, also, in a flask con-

taining an aqueous solution of 20 per cent potassium oxalate. Approximately 12 ounces of blood was added to one ounce of the oxalate solution to be used for inoculations. Total blood cell counts and hemoglobin determinations were made by the following method: 0.5 cc. of a 20 per cent solution of potassium oxalate was evaporated to dryness in an ordinary agglutination test tube. To this was added 3 cc. of uncoagulated blood. The two were then thoroughly though gently mixed. It was found unsatisfactory for counting purposes to fill the diluting pipettes at the time of necropsy. They were filled later with oxalated blood and fairly satisfactory counts were obtained. Toisson's fluid was used as a diluent. Whole blood was collected in sterile test tubes for serological examinations. Blood counts of five moose were made. The number of erythrocytes varied from 1,600,000 to 6,860,000; leucocytes, from 2,220 to 4,220; hemoglobin, 85 to 115 per cent. Hemoglobin determinations were made by either Tallquist's or Dare's hemoglobinometer. Differential leucocyte counts showed a wide variation. The lymphocytes varied from 9 to 55 per cent; polymorphonuclears, from 40 to 91 per cent; eosinophils, from 0 to 4 per cent; basophils, from 0 to 3 per cent, and monocytes from 0 to 2 per cent.

Table 2

Results of Blood Examinations

Moose No.	Date	Hemoglobin	Erythrocytes	Leucocytes	Lymphocytes, per cent	Polymorphonuclears, per cent	Eosinophils, per cent	Basophils, per cent	Monocytes, per cent	Pathological features
13	Mar. 21, 1933	115*	3,410,000	3,110	55	40	3	2	0	None
14	Apr. 10, 1933	85†	1,600,000	2,220	35	60	0	3	2	Basophilic stippling Secondary anemia
15	Sept. 12, 1933	100†	4,910,000	2,440	30	66	4	0	0	None
16	Oct. 11, 1933	90*	6,860,000	4,220	9	91	0	0	0	Neutrophilia
17	Jan. 12, 1934	95*	4,340,000	3,770	48	50	2	0	0	Slight anocytosis

* Tallquist method.

† Dare's hemoglobinometer.

Basophilic stippling of erythrocytes, in cases showing anemia, is the most striking pathological feature. The erythrocytes of five of seven moose included in the previous report (2) showed basophilic stippling, while only one moose (No. 14) included in this report showed the stippling. The ratio of cells showing the inclusion bodies is 1 to 250 or less. Some of the erythrocytes contained as high as 45 to 50 bodies, and they were not confined to a marginal location. A large majority of erythrocytes containing these bodies had 25 to 30 of them, and they

were rather evenly distributed throughout the cytoplasm of the cell. Smears made from the bone marrow of these cases showed erythrocytes with similar inclusion bodies. Properly stained blood smears failed to show evidence of blood parasites.

A summary of the results of the studies of the blood of these animals is given in Table 2.

SEROLOGICAL FINDINGS

The blood serum of each moose was examined serologically for the presence of specific agglutinins, with antigens prepared from *Bacterium tularense* and *Bacterium abortus*. No evidence of these diseases was found by this method.

BACTERIOLOGICAL FINDINGS

It is difficult to make bacteriological cultures under the conditions which exist where moose are found. Often it is either raining or snowing, or there is a high wind. Attempts no longer are made to culture the organs of the moose at the place of autopsy. The material to be cultured is placed in sterile cloth bags and taken to the nearest government or state-owned cabin. An ordinary gasoline blow torch is used to heat the instruments needed for preparing bacteriological cultures. Cultures were prepared from the lungs, livers, kidneys, spleens, brains, and heart's blood of the five moose. Five types of culture media were used in all these examinations; 10 per cent horse serum agar, cystine agar in infusion bouillon, plain broth, solidified blood serum, and liver-brain media. The organs were then brought to the laboratory and the cultures duplicated. Anaerobic cultures, using infusion agar and liver-brain media, were prepared which were incubated for at least 10 days. In practically all instances the cultures were sterile. In no instance were we able to isolate pathogenic bacteria.

Moose Nos. 13 and 14 were heavily infested with *Dermacentor albipictus* Packard. Moose No. 13 was infested chiefly with nymphal ticks. On March 23, 1933, the intestinal contents of 25 nymphal ticks were cultured. Before making the cultures, the ticks were washed for approximately three minutes in an alcoholic-bichloride solution (50 per cent alcohol and 1 to 1,000 bichloride). One tube of infusion bouillon was inoculated and one serum-agar slant was streaked with the intestinal contents of each tick. During the 10 days of incubation no bacterial growth developed. The intestinal contents of a second group of 25 adult and nymphal ticks were cultured. The same two media were used again. In this instance the ticks were not washed. A thin safety razor blade was heated, and while it was hot, each tick was cut longitudinally. A sterile platinum loop was then used to transfer the intestinal con-

tents to the media. Many of the cultures remained sterile. The cultures that produced organisms grew luxuriantly. The cultures were plated and three types of bacteria were isolated. A description of these organisms is omitted as they were found to be non-pathogenic for guinea pigs and rabbits.

Moose No. 14 was infested with thousands of adult ticks. Many of the females were partly engorged, while others were fully engorged. The intestinal contents of a group of 50 adult male and female ticks collected from this moose were examined bacteriologically. As before, the cultures that produced organisms grew luxuriantly, and the different colonies were sub-cultured until a pure culture was obtained. None of the cultures proved to be pathogenic for guinea pigs or rabbits.

Moose No. 12 had been dead for several days before it was shipped to the laboratory. It was infested with dead larval ticks. We were unable to isolate any pathogenic organism from these ticks by bacteriological examination.

Several thousand ticks were sent to two other laboratories expert in tick-culture technic. The results obtained by both laboratories were comparable to our own. The laboratories were unable to demonstrate that the ticks were the vectors of a pathogenic organism.

Cahn and his associates (4) report strikingly different results: "A bacterium, Klebsiella paralytica, has been isolated from ticks of this species taken from diseased moose. . . This organism, when injected into animals, has produced symptoms similar to those in the tick-infested animals and in the diseased moose. In practically every case, the injection of Klebsiella paralytica has proved fatal. It has been demonstrated that Klebsiella paralytica produces a highly potent toxic substance. This substance, when injected into animals, produces results identical with those obtained by culture inoculations."

We have not been able to obtain evidence supporting these conclusions. While reserving judgment, the question naturally arises as to whether or not these workers were dealing with an unusual infection not at all common to diseased moose. We were able in all cases to remove the ticks from the skin within 36 hours after the destruction of the moose. Post-mortem changes of the skin of a dead moose would occur naturally, unless precautions were taken to guard against it. It is not known whether ticks in contact with a partially decomposed skin could acquire an extraneous pathogenic organism.

EXPERIMENTAL ANIMAL INOCULATIONS

When first observed, Moose No. 13 held its head in an abnormal position. The following experimental animal inoculations were made: Two rabbits were inoculated intracranially with ¼ cc. of a saline sus-

pension prepared from the brain. Four guinea pigs were inoculated subcutaneously, two with 1 cc. and two with ½ cc. of a saline suspension prepared by macerating portions of skin that had been infested heavily with ticks. The lumbar region was the site of inoculation. Two guinea pigs were inoculated intraperitoneally with 2 cc. of diluted oxalated blood. Two guinea pigs were inoculated intraperitoneally with 2 cc. of a saline suspension prepared from the brain. Negative results were obtained.

One sheep was inoculated with ½ cc. of a saline suspension prepared from the brain. The inoculum was injected directly into the cerebrum through a trephine opening. The sheep was held under observation for 60 days. Negative results were obtained.

As soon as the material from Moose No. 14 was brought to the laboratory, it was used to repeat the experimental animal inoculations recorded for Moose No. 13. Negative results were obtained.

Material from the other three moose, (Nos. 15, 16, and 17) was brought to the laboratory to be used for inoculation purposes. A number of guinea pigs and rabbits were inoculated in each instance. Negative results were obtained.

TICK ATTACHMENT EXPERIMENTS

It was stated in our previous report (2) that attempts were made to have ticks attach to guinea pigs and rabbits. Those that did attach did not become engorged. An improved technic, described by Jellison and Philip (11), was used in these later studies. A copper-wire gauze cage was substituted for tin capsules. A coarse clipper was used to remove the hair before the cages were applied. Cloth skirts used to hold the ticks to the body were not so successful as the metal cages. Skin necrosis may develop, unless care is taken in applying the wire cages to the experimental animals.

Cahn and his associates (4) report: "Ticks (Dermacentor albipictus) taken from diseased moose and placed on animals in the laboratory have produced in these animals symptoms similar to those of moose disease."

Three hundred male and 300 unengorged female ticks, and 40 nymphal ticks were collected from Moose No. 13. Twenty ticks were placed on each of 28 guinea pigs. Wire cages were used to retain the ticks on 24 of the cavies. Only a few of the nymphal ticks, but all of the adults, attached. They remained attached for an average of 10 days. Eight of the females became fully engorged. The nymphal ticks that did attach moulted to adults. Four guinea pigs died during the experiment.

Twenty adult ticks (10 males and 10 females) were placed on each of four rabbits. All of the ticks attached. Two of the rabbits died. In

one instance death resulted from fighting; in the other, it resulted from fatty metamorphosis of the liver.

Two hundred seventy-four ticks, consisting of 18 engorged females, 140 unengorged females, and 116 males, were collected from Moose No. 14. From 10 to 13 ticks were placed on each of 21 guinea pigs. The engorged females did not attach. All of the adult ticks attached. The period of attachment varied from 7 to 16 days. Four of the guinea pigs died.

About 250 nymphal ticks were collected from Moose No. 17. Twenty were placed on each of 12 guinea pigs. Practically all of the ticks attached. In a sudden extreme change of temperature—to 20 degrees below zero—nine of the guinea pigs froze to death. The ticks were not removed from the frozen carcasses until 24 hours later. Only one-half of the ticks survived the exposure, and these were placed on six guinea pigs. The ticks attached again, and many moulted to adults. Eight of the ticks appeared to be fully engorged. The period of attachment exceeded one month in several cases. No deaths of guinea pigs, other than as stated above, have occurred.

Four guinea pigs, infested with ticks from Moose No. 13, died during the experiment. The vital organs of each guinea pig were subjected to a bacteriological examination and negative results were obtained. A saline suspension prepared from the heart's blood and spleen of each carcass was injected intraperitoneally in 1 and 2 cc. doses into two guinea pigs. The inoculated pigs were held under observation for 40 days and remained healthy.

Four guinea pigs, infested with ticks from Moose No. 14, died during the experiment. A thoro bacteriological examination was made of the vital organs of each carcass. In two instances negative results were obtained. *Escherichia communior* was isolated from the internal organs of one guinea pig. The prominent gross lesion of the fourth animal was pneumonia. Both lobes of the lungs were involved. Cultures prepared from the heart, liver, or spleen remained sterile. Cultures prepared from the lungs produced a gram negative coccus which grew readily on 10 per cent horse serum agar. This organism did not ferment dextrose, lactose, saccharose, mannite or maltose. A saline suspension of this organism was injected intraperitoneally into two guinea pigs. These animals were held under observation for six weeks. Negative results were obtained.

Our results are quite different from those obtained by Cahn and his associates (4). These authors describe the symptoms of diseased moose and also say that the laboratory animals on which ticks were placed showed symptoms similar to affected moose. The symptoms observed by us, as described, were quite different from those mentioned by these

authors. Also, the results of our studies of sick moose, which have been far more extensive than those of Cahn and his associates, lead us to doubt the pathological importance for moose of the organism isolated by these authors.

Blood Studies of Tick-Infested Experimental Animals

Blood studies were planned in order to observe any changes that might occur in the blood of experimental animals after they had become tick-infested. A preliminary blood examination was made of the guinea pigs, and only those with normal blood were used. The blood examination in all instances consisted in the following: hemoglobin determination, total erythrocyte and leucocyte counts, differential leucocyte counts, and blood smears were examined to ascertain whether any morphological changes of individual blood cells had occurred. After ticks were placed on the guinea pigs, the blood of each animal was examined weekly.

The blood of 58 guinea pigs was examined regularly at weekly intervals. It is not necessary to include data in relation to the different total blood counts or differential leucocyte counts. No evidence of anemia was found. Individual blood cells presented no pathological features. The blood contained no micro-organisms that could be demonstrated by any method known to us.

Here again Cahn and his associates (4) obtained different results. These authors state: "The blood picture of the diseased moose and that of the infected experimental animals were found to be similar, and all of these animals on which adult females of Dermacentor albipictus has engorged for over two days died."

HISTOPATHOLOGICAL EXAMINATION

Portions of various organs, especially of the central nervous system, were fixed in 10 per cent formaldehyde solution. Sections of the nervous system were fixed in alcohol. Tissues for microscopic examination were prepared and stained by different technics. The stains used included hematoxylin and eosin, hematoxylin and methylene blue, basic fuchsin, Gram-Weigart, and a special stain for the demonstration of Nissl's bodies. No significant pathological changes were observed in any of the tissues from five moose (Nos. 12, 13, 14, 16, and 18).

The splenomegaly of Moose No. 17 was the result of congestion. There was no evidence of a toxemia.

An important pathological change was found in the brain stem of Moose No. 15, the bull that carried his head in an abnormal position. The lesions consisted of a perivascular cuffing of lymphocytes around the arteries and veins. Around the larger blood vessels, the cuffing

was marked. There were no hemorrhagic areas in the brain stem. Sections from the cerebrum and cerebellum did not show any pathologic changes.

Significant pathological changes were found also in the brain stem of Moose No. 17. The lesions consisted of an extensive perivascular cuffing of lymphocytes around the arteries and veins. The lymphocytes were interspersed with a smaller number of erythrocytes and around these was an area of hemorrhage. Other areas showed evidence of hemorrhage only. Both types of lesions were rather diffusely scattered in only a limited part of the brain stem. Similar lesions were not found in the cerebrum and cerebellum. Meyer, Haring, and Howitt (10) described certain lesions found in the brain of horses affected with a neurotropic virus. The lesions found in the brain stem of these two moose correspond in some respects to those described by these authors. The interpretation of these abnormalities has been complicated by the finding of a small nematode parasite in the brain stem of Moose No. 17. The sections of the worm were of a uniform size, the greatest diameter being 238 microns and the smallest 192.4 microns. The cuticle is very thin

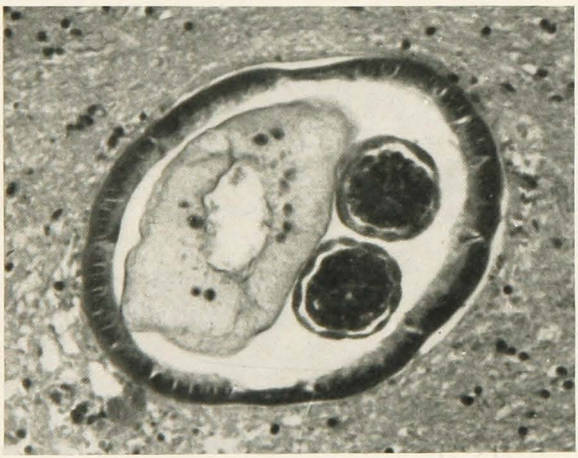

Fig. 1. Photomicrograph of a cross-section of the nematode found in the brain stem of Moose No. 17. (240 X) Note that cellular infiltration of the tissues surrounding the parasite is absent.

and the musculature poorly developed. The digestive tube and the two adjacent tubes of the reproductive organs in the body cavity of the parasite are shown in Figures 1 and 2. The reproductive tubes measure 57 microns in diameter. The worm was evidently immature and no characteristic structures that would aid in determining its identity were found. Immediately after the brain was removed from the cranial

Fig. 2. Photomicrograph of the same parasite shown in Fig. 1. (240 X)

cavity, it was placed intact in a 10 per cent formaldehyde fixative solution. After the fixation was completed, sections were taken from various parts of the brain for microscopic examination. The nematode was found in one of the small sections taken from the brain stem. Many serial sections of the latter were examined. Other sections from the brain stem were studied. The parasite was located in a small part of the pons. The posterior boundary of the area was about one-half inch anterior to the middle cerebellar peduncles. Serial sections gave no clue to the specific identity of this nematode parasite. So far we have been unable to examine the entire worm by the one method available. It would be difficult to recover such a small nematode intact from brain tissue by dissection methods. Examination of many serial sections of the brain stem tissue adjacent to the parasite did not show an extensive amount of cuffing and hemorrhage. There was no inflammatory reaction of the brain tissue immediately surrounding the nematode; nor was the parasite surrounded with a cellular infiltration. There is, of course, a possibility that this parasite was migrating through the brain tissue and that it had very recently invaded the area in which it was found. If so, it would be conceivable that the lesions observed in the brain tissue were caused by the parasite, and that the absence of definite lesions immediately adjacent to the parasite could be accounted for by the fact that the invasion of this particular area was recent.

DISCUSSION AND SUMMARY

We have reasons to believe that occasionally moose die as a result of mechanical injury. Moose No. 12 is an example. The carcass was shipped to the laboratory. It was reported that the animal had been observed wandering about in circles. Larval and nymphal ticks were found in the hair and on the skin; but all were dead. The autopsy revealed a severe contusion of the left frontalis muscle, and the frontal bone on the same side was fractured. After the muscle was removed, the fractured portions of the bone were removed without the aid of bone-cutting instruments. To those familiar with the habits of moose, it is surprising that more of them are not injured accidentally.

Opportunity has been afforded to observe five living sick moose and afterward to conduct autopsies of these animals. Moving pictures of each excellently portray their appearance, the symptoms, and many interesting characteristics. They also show some of the difficulties that one encounters while studying moose in their natural habitat.

Diseased moose usually show only a limited number of symptoms, among which are their lack of fear of man, their determined effort to remain within a small area, and their general weakened condition. Moose

No. 16 was practically blind, while No. 15 had impaired vision of one eye. The actions of the diseased moose, as described by Cahn and his co-workers (4) are not in accord with our observations. Diseased moose have never been observed to be constantly on the run or to race blindly through the woods. Instead, they have a tendency to remain within a rather limited area. Moose No. 13 was known to have remained constantly for two weeks within a somewhat circular area not more than five acres in extent. This could be determined easily by tracks in the snow. Moose are known to remain standing or lying down in one spot for several hours. When sick moose have been reported, we have found them one or two days later, with one or two exceptions, near the place where they were first observed. Those that had wandered were found nearby. These animals were in a weakened condition but were able to walk with an unsteady gait. We do not know just how long each moose would have lived if it had not been destroyed, but we believe that death would have occurred within a short time.

Our observations indicate that moose are not affected with a disease of seasonal periodicity. This is contrary to the statement of other authors (4). It is true that more dead and sick moose are found during the winter and early spring than during the remainder of the year. Autopsies have been made in every month of the year with the exception of July and August. Furthermore, it appears that heavy infestations with the tick, *Dermacentor albipictus* Packard, are not necessarily found on diseased moose.

The data show that the tick, *Dermacentor albipictus* Packard, has been found on its preferred host in the months from September to May, inclusive. The tick remains on the host for both moults. Ovipositing by the engorged female upon the hair or skin of live moose has not been observed, tho careful observations have been made.

In these studies (2, 5, and 8) the following animal parasites have been collected from the American moose, *Alces americanus*:

Cestoda

Cysticercus sp. (larval)
Taenia hydatigena Pallas (larval)
Echinococcus granulosus (Batsch) (larval)
Moniezia sp.[3]

Nematoda

Dictyocaulus hadweni Chapin
Nematodirella longispiculata York and Maplestone
Setaria labiato-papillosa Alessandrini[3]

[3] Reported for the first time for moose.

Trematoda

Fascioloides magna (Bassi)[4]
Peramphistomum cervi (Zeder)

Acarina

Dermacentor albipictus Packard

Diptera

Simulium venustum Say[4]

To this list must be added the unidentified nematode parasite found in the brain stem of one moose (No. 17).

Riley (13) reported the finding of the cysts of *Echinococcus granulosus* in six out of 13 moose examined for parasites. The author with others,[5] has examined 16 moose and found seven infestations. This large per cent of infestation resulted in a search to find, if possible, a reservoir of infection. On one of our trips to examine a moose, we were informed that a wolf had been trapped by a game warden. The carcass, the pelt removed, had been left in the woods. In order to obtain the carcass, it was necessary to travel 80 miles by car and four miles by canoe. The wolf was not infested with the adult Echinococcus. Later on, at our request, the district chief warden, Arthur Johnson, shipped two additional wolf carcasses to the laboratory. Both carcasses were heavily infested with adult Echinococcus. Riley (13) said: "Cooper Curtice, in 1892, notes '*Taenia echinococcus* von Siebold, very minute, found but once, in a dog killed at Washington, D.C., pound' . . . I inquired of Dr. M. C. Hall, Chief of the Zoological Division of the Bureau, and was informed that they had not encountered the adult worm except in experimental feedings."

We are unable to evaluate the pathological significance of the nematode found in the brain stem. Certainly the large eye worm would cause impaired vision, especially the one found in the posterior chamber of the eye. In this instance the parasite was entangled in a mass of tissue. Liver flukes can cause a great deal of damage to the infested organ, but the liver evidently has great power of tissue regeneration. Many deer livers, obtained from apparently healthy animals, have been examined. As many as 25 to 30 flukes have been recovered from a single liver. It is doubtful whether the liver fluke is an important etiological agent causing the death of moose. The presence of other internal parasites, with special reference to Echinococci and lung worms, do not account for all the symptoms. Nematodirella and Moniezia too have been found, and these, undoubtedly, play some part in emaciation and the

[4] Reported for the first time for moose.
[5] E. F. Waller, C. G. Fredine, F. G. Wallace and Wm. L. Jellison.

lack of thrift in the affected animals. One thing is certain, as a general rule moose are heavily parasitized.

The possibility that the tick may be a vector of some bacterial or protozoan disease is fully recognized, but the transmission of such an infection to guinea pigs and rabbits has failed.

Cahn and his co-workers (4) have stated: "While we have not proved that Klebsiella paralytica is the cause of moose disease, we have presented a series of observations which strongly indicate that it may be the cause." These authors, by taking ticks from a dead moose and placing them on animals in the laboratory, were able to produce in these animals symptoms similar to those which had been observed by them in moose. The same authors isolated a bacterium, *Klebsiella paralytica*, from the intestinal contents of an engorged adult female *Dermacentor albipictus*. This organism, when injected into animals, produced symptoms similar to those in the tick-infested animals and in the diseased moose. Furthermore, they present evidence that this organism produces a highly toxic substance.

In our own experiments we have removed the ticks from the skin within 36 hours after the destruction of moose. In one instance it is known that the piece of skin which these investigators (4) received was removed from an animal that had been found dead.

We have outlined in detail the experiments that were conducted by placing ticks on laboratory animals. Our results were negative in producing any symptoms of disease. The deaths that did occur have been mentioned. The tick-infested guinea pigs often became emaciated, but that is not surprising, because they constantly tried to free themselves of the parasites. At the present time we have guinea pigs that have been tick-infested for two months. Some of the ticks have been engorging for one month.

The blood of the tick-infested guinea pigs has been examined regularly and no pathological changes have been found.

Various organisms that were non-pathogenic to guinea pigs have been isolated from the intestinal contents of the tick, *Dermacentor albipictus* Packard. All of the organisms isolated have been sub-cultured regularly. They continue to be avirulent to guinea pigs.

The results of blood examinations of the moose have been largely negative. Basophilic stippling of erythrocytes in conjunction with a secondary anemia is the only pathologic feature that has been observed. The significance of the inclusion bodies found in the blood cells of animals affected with other diseases is not understood entirely. They may prove to be of greater importance, when more is known about these intracellular structures. Eosinophilia has not been found even in those animals infested heavily with cestodes.

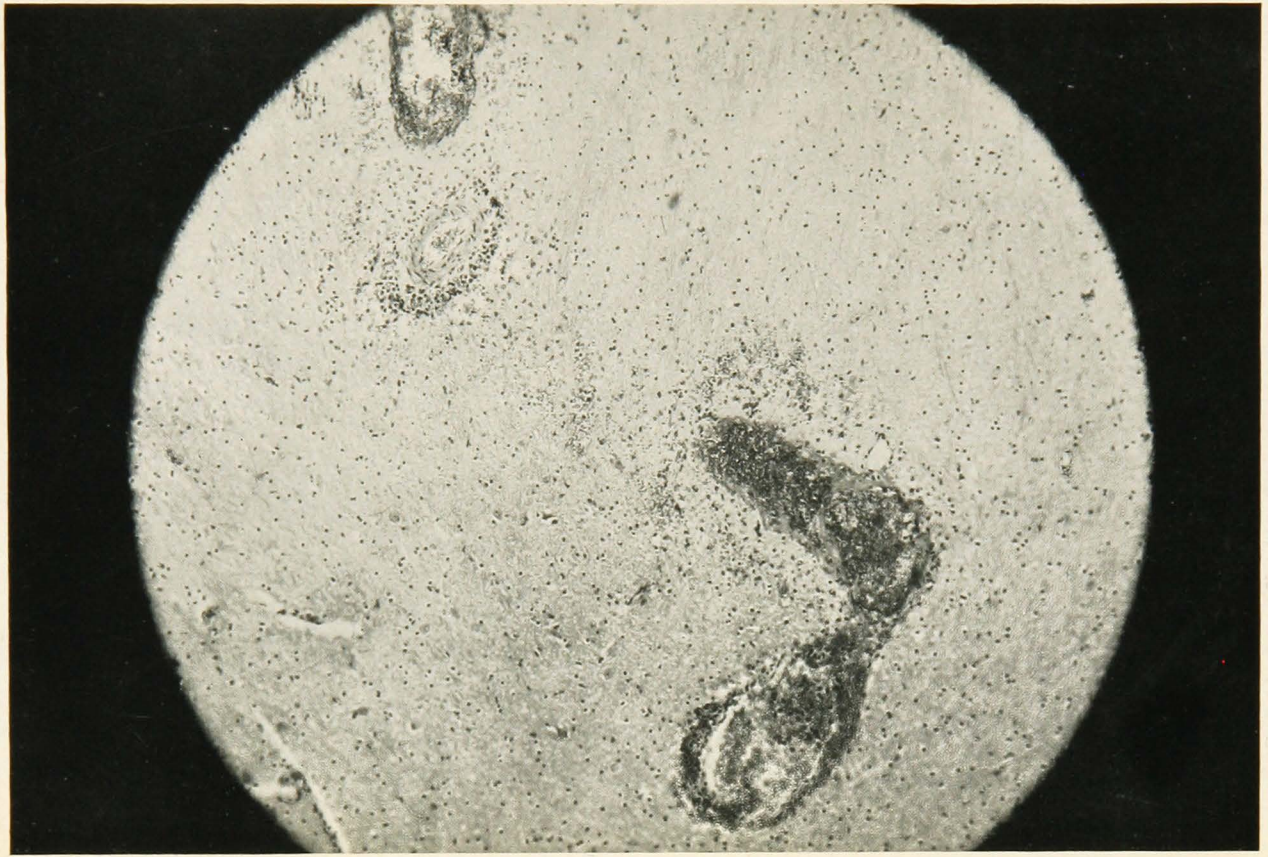

Fig. 3. Photomicrograph of brain stem of Moose No. 17. (90 X) Cuffing of lymphocytes around the blood vessels is shown.

Negative results were obtained by inoculating experimental animals with saline suspensions prepared from the different vital structures of moose. The basal encephalitis that has been found in two moose (Nos. 15 and 17) is of pathologic importance (Figure 3). The perivascular cuffing of lymphocytes and hemorrhage were confined to only a part of the brain stem. Similar lesions were not found in the cerebrum or cerebellum. It is difficult to interpret the significance of the basal encephalitis in terms of pathological interference with nerve impulses transmitted through the brain stem. It is reasonable to suppose that an encephalitis could be responsible for certain of the symptoms observed and also for death. The role of the nematode parasite found in Moose No. 17 is not clear. Experimental animals were not inoculated with brain material from this moose, and, therefore, we do not have any data regarding the nature of the etiological agent. The latter remains to be demonstrated.

Our knowledge of the diseases that affect wild animals under natural conditions is limited. We are more convinced than before (2), despite the evidence presented by other authors (4), that the losses of moose that occur in Minnesota are not the result of a single pathogen, also that the conditions studied are brought about through factors co-operating to reduce the vitality of the moose.

LITERATURE CITED

1. Surber, Thaddeus. The mammals of Minnesota. Minnesota Game and Fish Department, St. Paul, Minnesota. 1932.
2. Fenstermacher, R., and Jellison, W. L. Diseases affecting moose. Minnesota Agr. Expt. Sta. Bull. 294. 1933.
3. Howard, C. W. New tick records for Minnesota. Jour. Econ. Ent. **10**:560. 1917.
4. Wallace, G. I., Cahn, Alvin R., and Thomas, Lyell J. *Klebsiella paralytica:* A new pathogenic bacterium from "moose disease." Jour. Inf. Dis., **53**: (3):386. 1933.
5. Wallace, F. G. Parasites collected from the moose, *Alces americanus* in Northern Minnesota. Jour. Am. Vet. Med. Assn., N. S. **37**(5):770. 1934.
6. Kaupp, B. F. Animal parasites and parasitic diseases, p. 95. Alexander Eger Co., Chicago, Ill. 1914.
7. Law, Ronald G., and Kennedy, Arnold H. *Echinococcus granulosus* in a moose. North Amer. Vet., **14**(10):33-34. 1933.
8. Jellison, W. L. Further studies on the parasites of large game animals in Northern Minnesota. A thesis submitted to the Faculty of the Graduate School, University of Minnesota. December, 1933.
9. Boulenger, C. L. On some filariid parasites of cattle and other ruminants. Parasitol. **12**:341-49. 1920.

10. Meyer, K. F., Haring, C. M., and Howitt, B. Newer knowledge of the neurotropic virus infections of the horse. Jour. Am. Vet. Med. Assn., N. S. **32**(3):376. 1931.
11. Jellison, W. L., and Philip, C. E. Technique for routine and experimental feeding of certain Ixodid ticks on guinea pigs and rabbits. Pub. Health Report. **48**(35):1081. 1933.
12. Dikmans, G. *Nematodirella longispiculata* from the moose. Jour. Parasitology, **18**:303. 1932.
13. Riley, Wm. A. Reservoirs of Echinococcus in Minnesota. Minnesota Medicine, **16**:744. December, 1933.
14. Ransom, B. H. *Cysticercus ovis,* the cause of tapeworm cysts in mutton. Jour. of Agricultural Research, **1**(1):15-58. 1913.